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ENERGY METABOLISM OF THE ISOLATED PERFUSED RAT BRAIN AS INFLUENCED BY ANESTHETICS

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Abstract—The isolated perfused rat brain was used for a comparative study of the effects of thiopental (0·2 mM), hexobarbital (0·2 mM), γ-hydroxybutyric acid (4 mM) and ketamine (0·05 mM) on cerebral energy metabolism. After a perfusion period of 30 min the brain levels of high-energy phosphates, as well as those of substrates and metabolites of the glycolytic pathway were measured spectrophotometrically. All drugs tested caused an increase in cerebral P-creatine. Furthermore, after perfusion with the barbiturates, or γ-hydroxybutyric acid, increased levels of glycogen and glucose, and decreased levels of pyruvate and lactate were measured. After ketamine perfusion, however, the glycogen and glucose levels tended to be diminished. Doubling the glucose concentration in the perfusion medium led to a marked increase in brain glucose, which after anesthesia with thiopental increased even further.

It is well established that the principal source of energy for normal brain function is derived from the metabolic utilization of glucose. Numerous investigations have shown that anesthetics may affect cerebral energy metabolism, in particular the levels of high-energy phosphates [1, 2], glycogen and glucose [3, 4] and lactate and pyruvate [5, 6]. However, there is a large number of agents which induce anesthesia. The chemical structure of these substances is very different, and consequently, it is of interest to investigate whether different anesthetics produce comparable changes in brain metabolism.

Comparative studies on the effects of anesthetics on cerebral metabolism *in vivo* have already been performed [1, 7, 8]. Investigations on animals *in vivo* however, cannot avoid the central effects of a drug being influenced by its metabolites formed in the liver or being superimposed by secondary peripheral actions. An isolated perfused brain preparation overcomes these disadvantages, and has been shown to be a valuable model for studying drugs acting on the CNS [9]. We have used this system for a comparative study of the effects of various intravenous anesthetics on cerebral energy metabolism.

METHODS

Materials. Male Sprague–Dawley rats weighing 200–300 g and fed on a standard diet (Altromin®) and tap water were used. Biochemicals and enzymes were bought from Boehringer, Mannheim, bovine serum albumin was purchased from Behring-Werke, Marburg (quality: trocken, 'reinst'). The drugs used in this study were thiopental (Promonta, Hamburg), hexobarbital (Evipan®-Natrium; Bayer, Leverkusen), γ-hydroxybutyric acid, sodium salt (Somsanit®; Köhler-Chemie, Alsbach), ketamine (Ketanest®; Parke-Davis, München), and urethan (Merck, Darmstadt). All other chemicals were of reagent grade.

Preparation and perfusion of the brain. Preparation of the isolated rat brain according to Andjus *et al.* [10] was performed under urethan anesthesia (1·2 g/kg intraperitoneally) without interruption of the circulation. As described in previous papers [11, 12, 13], closed-circuit perfusion was carried out with 100 ml of perfusion medium at a constant arterial pressure of 100–120 mm Hg. The perfusion rate was 3–5 ml/min and remained unchanged within each experiment. Bipolar EEGs were recorded from each side of the brain [14]. After a perfusion time of 30 min the brain preparations, still supplied with the perfusion medium [15], were plunged into a Dewar flask containing liquid nitrogen. The frozen brains were removed and extracted with 0·6 N perchloric acid (for details of procedure, see Krieglstein *et al.* [11]).

The perfusion medium contained 2% (w/v) bovine serum albumin, 28·2 ± 0·6% (n = 30) washed bovine red cells and about 7·5 mM glucose in Krebs–Henseleit-saline. The drugs to be studied were added to the medium to give the following concentrations: thiopental 0·2 mM, hexobarbital 0·2 mM, γ-hydroxybutyric acid 4 mM, and ketamine 0·05 mM. The perfusion experiments were carried out with each of these drugs. Experiments with a medium without a drug were used as controls. Furthermore, a series of experiments with thiopental (0·2 mM) in a perfusion medium containing *ca* 14 mM glucose was carried out.

Analytical methods. The following substrates and metabolites in the isolated brain were estimated according to the methods reported by Bergmeyer [16]: P-creatine, ATP, ADP, AMP, glycogen, glucose, glucose-6-P, fructose-6-P, pyruvate, lactate, α-ketoglutarate, glutamate, and ammonia. All enzymic measurements were made at 340 nm and 25°, using a Zeiss PMQ II spectrophotometer with an attached Servogor recorder. The values given as means ± S.E.M. in μmoles/g brain wet weight were not corrected for the

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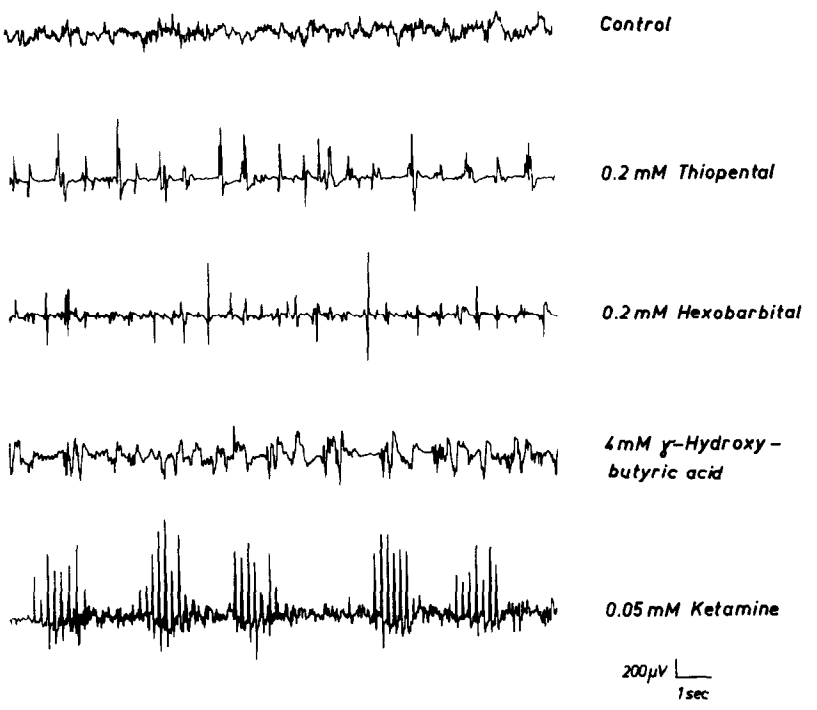

Fig. 1. EEGs of the isolated rat brain as influenced by various anesthetics in the perfusion medium. The above recordings were obtained after a perfusion period of 30 min. Bipolar leads in the parietal region of both hemispheres were used. Since the tracings are usually symmetrical and synchronous only one EEG channel for each drug is shown. An EEG recording of an experiment performed without a drug in the perfusion medium is given as a control.

extracellular space. Glycogen was calculated as glucosyl units (1 μmole being equivalent to 162 μg). Student's *t*-test was used to determine the significance of differences between mean values.

RESULTS

The drugs were used in concentrations similar to those determined in the blood of human patients or laboratory animals during anesthesia with the corresponding drug [17–20]. They induced EEG patterns characteristic of anesthesia (Fig. 1). Thus, the recordings obtained under thiopental, hexobarbital or γ-hydroxybutyric acid showed a decreased frequency with prevailing slow waves in the theta and delta range interrrupted by isolated spike discharges [21, 22]. Ketamine, however, induced a background activity of enhanced low-voltage fast waves which was interrupted regularly by bursts of high-amplitude activity composed of typical spike-and-wave complexes [23].

The substrate and metabolite levels measured in the isolated rat brain are summarized in Table 1. Thiopental, hexobarbital, γ-hydroxybutyric acid as well as ketamine caused an increase in cerebral P-creatine, but did not markedly change the levels of adenine nucleotides. The glycolytic pathway was comparably influenced only by the barbiturates and γ-hydroxybutyric acid. In these groups glycogen levels were a little higher than in control experiments. Cere-

Table 1. Substrate and metabolite levels in the isolated rat brain after perfusion with various anesthetics for 30 min

	Control	Thiopental 0·2 mM	Hexobarbital 0·2 mM	γ-Hydroxybutyric acid 4 mM	Ketamine 0·05 mM
P-Creatine	2·67 ± 0·05	3·25 ± 0·15†	3·07 ± 0·11†	2·98 ± 0·11*	2·91 ± 0·08*
ATP	2·06 ± 0·04	2·20 ± 0·09	2·04 ± 0·08	2·07 ± 0·09	2·24 ± 0·07
ADP	0·68 ± 0·06	0·65 ± 0·03	0·66 ± 0·03	0·49 ± 0·04*	0·63 ± 0·05
ATP/ADP	3·2 ± 0·4	3·4 ± 0·2	3·1 ± 0·1	4·4 ± 0·3*	3·7 ± 0·4
AMP	0·26 ± 0·02	0·19 ± 0·02*	0·21 ± 0·02	0·20 ± 0·01*	0·23 ± 0·02
ATP + ADP + AMP	3·01 ± 0·06	3·04 ± 0·12	2·91 ± 0·11	2·76 ± 0·12	3·10 ± 0·09
Glycogen	1·87 ± 0·27	2·32 ± 0·15	2·35 ± 0·11	2·17 ± 0·26	1·69 ± 0·09
Glucose	1·21 ± 0·06	1·78 ± 0·11†	1·80 ± 0·08†	1·62 ± 0·08†	1·00 ± 0·10
Glucose-6-P	0·062 ± 0·005	0·064 ± 0·006	0·056 ± 0·002	0·063 ± 0·006	0·061 ± 0·007
Fructose-6-P	0·015 ± 0·001	0·015 ± 0·001	0·015 ± 0·001	0·015 ± 0·001	0·012 ± 0·001
Pyruvate	0·202 ± 0·018	0·155 ± 0·024	0·174 ± 0·016	0·166 ± 0·024	0·239 ± 0·017
Lactate	4·71 ± 0·53	3·28 ± 0·47	3·79 ± 0·47	3·22 ± 0·67	5·32 ± 0·32
Lactate/Pyruvate	23·1 ± 0·8	21·5 ± 1·2	21·6 ± 1·5	18·7 ± 1·7*	22·5 ± 1·1

Values (except ratios) are μmoles/g brain (wet wt), means ± S.E.M., n = 6.
* $P < 0.05$ compared with controls.
† $P < 0.01$ compared with controls.

Table 2. Substrate and metabolite levels in isolated rat brain after perfusion with thiopental and two different glucose concentrations

	7·5 mM glucose in the medium		14 mM glucose in the medium	
	Control n = 6	0·2 mM Thiopental n = 6	Control n = 4	0·2 mM Thiopental n = 4
P-Creatine	2·78 ± 0·16	3·47 ± 0·21*	2·86 ± 0·15	3·47 ± 0·11*
ATP	2·18 ± 0·04	2·20 ± 0·05	2·32 ± 0·03	2·32 ± 0·04
ADP	0·79 ± 0·05	0·58 ± 0·03†	0·69 ± 0·04	0·69 ± 0·03
ATP/ADP	2·8 ± 0·2	3·9 ± 0·3*	3·5 ± 0·2	3·4 ± 0·1
AMP	0·20 ± 0·04	0·22 ± 0·02	0·23 ± 0·01	0·15 ± 0·04
ATP + ADP + AMP	3·18 ± 0·05	2·99 ± 0·04	3·24 ± 0·03	3·16 ± 0·05
Glycogen	2·23 ± 0·07	2·44 ± 0·21	2·27 ± 0·37	−2·71 ± 0·17
Glucose	1·39 ± 0·14	1·83 ± 0·08*	2·63 ± 0·20	3·38 ± 0·19*
Glucose-6-P	0·071 ± 0·005	0·090 ± 0·010	0·069 ± 0·008	0·063 ± 0·005
Fructose-6-P	0·016 ± 0·001	0·017 ± 0·002	0·014 ± 0·002	0·015 ± 0·001
Pyruvate	0·191 ± 0·015	0·131 ± 0·017*	0·198 ± 0·027	0·131 ± 0·005
Lactate	4·04 ± 0·49	2·45 ± 0·26*	4·23 ± 0·89	2·68 ± 0·17
Lactate/Pyruvate	21·1 ± 2·1	19·3 ± 1·4	20·8 ± 1·5	20·6 ± 1·4
α-Ketoglutarate	0·069 ± 0·009	0·071 ± 0·007	0·065 ± 0·004	0·066 ± 0·006
Glutamate	10·34 ± 0·90	9·86 ± 0·53	9·52 ± 0·25	11·46 ± 1·13
Ammonia	0·48 ± 0·08	0·57 ± 0·15	0·47 ± 0·12	0·65 ± 0·13

All values (except ratios) are μmoles/g brain (wet wt), means ± S.E.M.
Significance of difference from corresponding control: * $P < 0.05$; † $P < 0.01$.

bral glucose concentrations, however, were significantly increased. In contrast with these drugs ketamine caused no rise in glycogen or glucose levels; the values obtained tended to be even lower than control values. The hexose phosphates remained unchanged in all groups. Furthermore, there was a tendency towards lower lactate and pyruvate levels and lower lactate/pyruvate ratios only after the barbiturates or γ-hydroxybutyric acid.

When the glucose concentration of the perfusion medium was doubled from about 7·5 mM to 14 mM (Table 2) higher levels of brain glucose were measured in control experiments as well as in experiments with thiopental. The levels of glycogen and the glycolytic intermediates were not influenced. After perfusion with 14 mM glucose the same metabolic alterations caused by thiopental were observed as with 7·5 mM glucose (see also Table 1). In particular the concentration of brain glucose, already elevated by the use of a perfusion medium containing a higher glucose concentration, was further elevated by thiopental.

DISCUSSION

At present there is only little knowledge of how the accumulation of high-energy compounds is connected with anesthesia. Essentially, three possibilities may be considered. The increased amount of high energy compounds could be a result of anesthesia. As a consequence of depressed CNS activity cerebral energy demand is reduced and therefore utilization of cellular energy stores will be diminished. Furthermore, suppression of cerebral metabolism by anesthetics could be connected with the formation of anesthesia. Finally, metabolic alterations could be side effects of anesthetics, which are unspecific and not correlated with the anesthetic action of these drugs. It has previously been thought most probable that the increase of high-energy compounds is secondary to anesthesia. It might therefore be expected that changes of cerebral metabolism should be uniform after the application of various different anesthetics.

Elevated brain levels of high-energy phosphates, especially of P-creatine, glycogen and glucose and decreased levels of lactate and pyruvate, as observed with the barbiturates and γ-hydroxybutyric acid, have been found also in the isolated rat brain after phenobarbital [11], chloral hydrate and trichloroethanol [15] perfusion as well as in the brain of animals *in vivo* after treatment with various anesthetics [1–8, 24, 25]. Fleming and Lacourt [24] have reported unchanged P-creatine levels, but a marked increase in ATP after administration of γ-hydroxybutyric acid. Although the higher P-creatine levels found here seem to confirm previous suggestions that anesthesia is associated with an increase of high-energy compounds, it cannot be ruled out that the differences observed may be partly due to a protective effect of anesthetics against autolytic postmortem changes occurring before all layers of the brain tissue are fixed by being frozen [26].

The metabolic alterations measured after anesthesia with ketamine are partly in contrast with those mentioned above. P-creatine also was elevated, but glycolysis seemed to be enhanced rather than depressed. This might be a consequence of excitation effects of this drug, which are also evident in the EEG. Neither with phenobarbital nor with the drugs investigated in this study were the levels of glucose-6-P in the isolated brain changed as was the case with chloral hydrate, trichloroethanol [15] or several tricyclic psychoactive drugs [27]. After barbiturate treatment of rats and mice *in vivo* both increases [1] as well as decreases [28] of glucose-6-P have been reported. Leonard and Watkinson [29] found increased glucose-6-P levels after only a sedative dose of γ-hydroxybutyric acid suggesting an increase in hexokinase activity. In another study this drug has been shown to enhance the activity of glucose-6-P dehydrogenase in brain [25].

Since the anesthetics investigated did not always cause an accumulation of the same high-energy compounds, the possibility cannot be ruled out that alterations of energy metabolism occur prior to anesthesia. They might even cause anesthesia. On the other hand anesthetic drugs of various chemical and physical properties may have different sites of action in the brain. By measuring substrate levels as average values

for the whole brain regionally specific metabolic changes may overlap and simulate varying drug effects.

The most striking effect of anesthetics on cerebral metabolism is the marked increase in glucose. Mayman and co-workers [3] found a strong correlation between brain and blood glucose levels following the injection of phenobarbital. We have shown that the concentration of glucose was elevated in the isolated brain when the glucose concentration in the perfusion medium was increased; in spite of this, thiopental produced a further increase in the already increased glucose content of the brain. Therefore we can exclude the possibility that the rise in brain glucose after anesthetics is produced by an increase in blood glucose. Moreover, work by Taberner [30] has shown that the accumulation of cerebral glucose after the administration of γ-hydroxybutyric acid or barbiturates cannot be explained in terms of an increased net uptake of glucose into brain. The influence of glucose upon effects of anesthetics on cerebral energy metabolism has to be the subject of a further investigation.

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